



DAVID B. WILSON, Ph.D.
Professor, Biochemistry, Molecular & Cell Biology
Department of Molecular Biology & Genetics
458 Biotechnology Building, Ithaca, NY 14853, USA

CORNELL UNIVERSITY

Phone: (607) 255-5706
Fax: (607) 255-2428
E-mail: dbw3@Cornell.edu

July 25, 2000

Dr. Michael E. Himmel
Biotechnology Center for Fuels and Chemicals
National Renewable Energy Laboratory
1617 Cole Boulevard
Golden, CO 80401
Email: mike_himmel@nrel.gov

Re: Progress Report for #XDH-9-29048-01

Dear Mike,

Here is the report letter for subcontract #XDH-9-29048-01 covering the period from January 18, 2000 to July 21, 2000. A major activity in this period has been the development of a new substrate that detects crystalline cellulase activity in an agar overlay, either from single colonies or enzyme bands on a gel. The assay is run in the same way as a CMC overlay assay, but it requires much longer incubation times as the substrate is crystalline cellulose. This assay will be useful in screening for new cellulolytic microorganisms, in screening gene libraries for cellulase genes that are active on crystalline cellulose, and for directed evolution of cellulase genes to achieve higher activity on crystalline cellulose. Although I still believe that national design will ultimately give the most active cellulases, there is a place for directed evolution now that there is an assay for screening for more active mutants.

The substrate we have prepared is ball milled bacterial microcrystalline cellulose. We have shown that this material shows strong synergism when we use an endo-exocellulase mixture, so that it is detecting crystalline cellulase activity. Ball milling produces a material that gives a uniform suspension that forms a uniform turbid background on a plate. We tested *Streptomyces lividans* colonies that contained the E4 gene, and ones that contained both the E2 and the E3 genes, as well as control strains that did not contain a cellulase gene. We were able to see clearing only around the cellulase-producing colonies after a 24 hour incubation at 50°C. We stained the plates with Congo red after a two day incubation and there was a yellow zone around each cellulase-producing colony, but not around the control colonies. My first attempt to detect activity in *E. coli* clones failed as no clearings were seen even after a four day incubation. There are two things to test before I conclude that activity in *E. coli* can not be detected. One is to lyse the cells, since in *E. coli* most of the enzyme is trapped inside the outer membrane where it is not accessible to the cellulose. The other is to induce the synthesis of the cellulase by exposing the colonies to IPTG for several hours before adding the overlay. Even if the assay is not sensitive enough to detect activity in *E. coli* transformants, it is possible to carry out directed evolution in *S. lividans*, although it is more difficult.

Another project we are starting is to prepare selenomethionine-labeled E3cd to send to Joshua Sakon to allow him to complete its structure. Unfortunately, *E. coli* does not express E3cd,

Dr. Michael E. Himmel

Page 2

July 25, 2000

even though it does express E3. Since the E3cd we produce from E3 by protease treatment has not crystallized, we will try to use a methionine requiring *S. lividans* mutant to produce labeled E3cd.

The last project has been continuing our study of the mechanism of endocellulase E2. John Brady's modeling experiments suggested that Arg78 may be important for catalysis. Therefore, we are preparing two Arg78 mutants, one with Ala and one with Lys in position 78. The Ala mutation has been produced and it has very low activity on a CMC overlay suggesting that this residue is important for activity. However, we need to sequence the plasmid to make certain there are no extra mutations and then produce some pure enzyme to fully characterize its properties. We have also isolated Ala mutations in two E2 Ser residues (84 and 85). Ser181 in CBHII was suggested to be hydrogen bonded to the catalytic water molecule, but the equivalent residue in E2 Ser85 can be replaced by Ala and the mutant enzyme has high activity on a CMC overlay, as does the Ser84Ala mutant enzyme. Thus, I do not think that this is the catalytic water molecule. A recent paper (Zou et al., Structure 7: 1035-1045), reports that there is another possible catalytic water molecule in CBHII, which is closer to the cleaved bond than the one interacting with the serine residue. Our results suggest this water molecule is more likely to be the catalytic water molecule.

Sincerely yours,

David

David B. Wilson, Ph.D.
Professor of Biochemistry,
Molecular and Cell Biology

DBW/ves

Cc: pat_weitzel@nrel.gov
lyn_lumberg@nrel.gov